

# Resurrection of *Neocardiochiles* Szépligeti, 1908 (Hymenoptera, Braconidae, Cardiochilinae) with descriptions of five new species from the Neotropical region

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Academic editor: Jose Fernandez-Triana | Received 5 April 2022 | Accepted 15 May 2022 | Published 30 June 2022

<http://zoobank.org/410351D0-DE45-4C43-A31E-35DC8DB55B62>

**Citation:** Kang I, Whitfield JB, Owens BE, Chen J (2022) Resurrection of *Neocardiochiles* Szépligeti, 1908 (Hymenoptera, Braconidae, Cardiochilinae) with descriptions of five new species from the Neotropical region. Journal of Hymenoptera Research 91: 41–68. <https://doi.org/10.3897/jhr.91.84937>

## Abstract

*Neocardiochiles* Szépligeti, 1908, is a rare Neotropical genus of the subfamily Cardiochilinae Ashmead, 1900. The genus was previously synonymized with *Heteropteron* Brullé, 1846 by Dangerfield et al. (1999). In this study, we examined multiple specimens of *Heteropteron*-related genera: *Heteropteron*, *Neocardiochiles*, and *Wesmaelella* Spinola, 1851, and resurrect *Neocardiochiles* as a valid genus based on morphological data. As a result, five new species, *N. alexeyi* Kang, **sp. nov.** from Ecuador, *N. franki* Kang, **sp. nov.** from Costa Rica, *N. braeti* Kang, **sp. nov.**, *N. chriscarltoni* Kang, **sp. nov.**, and *N. victoriae* Kang, **sp. nov.**, from French Guiana are included as members of *Neocardiochiles* and described based on morphological and molecular data. Additionally, four species previously included in *Heteropteron* are transferred to *Neocardiochiles*: *Neocardiochiles fasciipennis* Szépligeti, 1908, **comb. nov.**, *Neocardiochiles hasegawai* (Dabek & Whitfield, 2020) **comb. nov.**, *Neocardiochiles kidonoi* (Dabek & Whitfield, 2020), **comb. nov.**, and *Neocardiochiles whitfieldi* (Mercado, 2003), **comb. nov.**. Diagnosis of each taxon and both traditional and interactive identification keys to *Neocardiochiles* species are included. Molecular data of *N. alexeyi* **sp. nov.**, *N. chriscarltoni* **sp. nov.**, *N. victoriae* **sp. nov.**, and *N. hasegawaii* (Dabek & Whitfield, 2020), are also provided.



**Keywords**

Delta, DNA, Intkey, parasitoid wasps, phylogeny, taxonomy, 16S rRNA, 28S rRNA

**Introduction**

Members of the cardiochiline genera, *Heteropteron* Brullé, 1846, *Neocardiochiles* Szépligeti, 1908 and *Wesmaelella* Spinola, 1851 are rarely-collected braconid wasps in the Neotropical region, which possess a relatively large body size (6.5–13 mm). These three genera have been treated as the most plesiotypic members of the Cardiochilinae Ashmead, 1900 based on morphological characters (Dangerfield et al. 1999). *Heteropteron* was confirmed as an early-diverging genus based on molecular data (Murphy et al. 2008). Prior to the current publication, only seven species were recorded from the three genera, including two recently described species: *N. hasegawai* (Dabek & Whitfield, 2020), comb. nov. and *N. kidonoi* (Dabek & Whitfield, 2020), comb. nov.. Detailed biological information for these two species was collected by Drs Daniel Janzen and Winnie Hallwachs and their team members at the Area de Conservación Guanacaste (ACG) (Dabek et al. 2020).

Despite the rarity of the specimens of the three genera in museum collections, their relationships have been frequently discussed and have fluctuated even in recent decades (Dabek et al. 2020). All three genera were considered valid by Whitfield and Dangerfield (1997), but Dangerfield et al. (1999) later subsumed *Neocardiochiles* and *Wesmaelella* into *Heteropteron*. Mercado and Wharton (2003) partly agreed with these synonymies, resurrecting *Wesmaelella* as a valid genus while retaining *Neocardiochiles* as a junior synonym of *Heteropteron* based on wing venation. Papp (2014) and Dabek et al. (2020) further validated separation of *Heteropteron* and *Wesmaelella* before the current study. Herein, we resurrect *Neocardiochiles* as a valid genus based on a morphological analysis of previously recorded and new species. In addition, both traditional and interactive keys to species of *Neocardiochiles* are given for identification, and molecular data for four species of *Neocardiochiles* are included.

**Materials and methods****Specimen information**

The type specimens of recorded species for this work were borrowed from the Hungarian Natural History Museum (HMNH; Budapest, Hungary), and a type specimen of *N. whitfieldi* (Mercado, 2003), comb. nov. was examined at the Texas A&M University Insect Collection (TAMU; College Station, Texas, USA) by the first author. Two non-type specimens of *N. kidonoi* and *N. hasegawai* reared at ACG were also included and examined. Other specimens were provided by the Hymenoptera Institute (HIC; 116 Franklin Ave., Redlands, California, USA), Illinois Natural History



Survey (INHS; Champaign, Illinois, USA), University of Wyoming Insects Museum, (Laramie, Wyoming, USA), and Mr Yves Braet's private collection. Holotypes of new species will be deposited in Canadian National Collection of Insects (CNC; Ottawa, Ontario, Canada), UWIM, and the Royal Belgian Institute of Natural Sciences (RBINS; Brussels, Belgium).

## Morphological analyses

A Leica MZ75 stereomicroscope was used to examine specimens. The morphological terms are mostly based on Dangerfield et al. (1999) and Sharkey and Wharton (1997). Terms for sculpturing follow Harris (1979). The following acronyms are used throughout: POL (distance between posterior ocelli), T1 (first metasomal tergum), T2 (second metasomal tergum), and T3 (third metasomal tergum). Color images were taken using a Visionary Digital BK Plus imaging system (Dun, Inc.) equipped with a Canon EOS 5DS DSLR. Captured images were stacked via Zerene Stacker v.1.04 (Zerene Systems LLC.), and stacked images were edited in Adobe Photoshop CS 6 and Photoshop CC 2022 v. 23.0 (Adobe Systems, Inc). Image plates were produced using Photoshop CC 2022 v. 23.0. Using the Atlas of Living Australia (ALA) version of DELTA Editor (Open DELTA 1.02 beta) (Dallwitz et al. 1999), morphological characters of the members of *Neocardiochiles* were recorded, and an interactive key was generated. The interactive key was tested using Intkey (Dallwitz et al. 2000). Morphometric characters were measured via the same Adobe software. The numbers in parentheses in species descriptions indicate the actual size of body parts, and the unit of length is mm.

## Molecular analyses

DNA was extracted from one to two legs of each specimen using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). Mitochondrial 16S rRNA (16S), and nuclear 28S rRNA (28S) genes were targeted and amplified using the primers listed in Table 1. PCR reaction volumes were 25 µl containing 12.5 µl of DreamTaq Green PCR Master Mix (2X) (Thermo Scientific), 1–2 µl of template genomic DNA, 9.5 µl of ddH<sub>2</sub>O, and 1.0 µl of each primer at 5–10 µM. PCR conditions were 95 °C for 3 min; 40 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min; and a final extension at 72 °C for 7 min for 16S. For 28S, we followed Smith et al. (2008), but reduced the cycle number from 35 to 30. PCR products were visualized on a 1.8–2.0% agarose gel to confirm the success of amplification. PCR products were initially cleaned by a EtOH clean-up method and sequenced on the 3130xl Genetic Analyzers (Applied

**Table 1.** Information for genes and primers used in the current study.

Target genes	Primer sequences (5'→3')	References
16S rRNA	cacctgtttatcaaaaacat (F)	Dowton and Austin (1994)
	cttaattcaacatcgaggtc (R)	Chen et al. (2006)
28S rRNA	agagagagttcaagagtacgtg (F)	Belshaw and Quicke (1997)
	ttggtccgtgtttcaagacgg (R)	Campbell et al. (2000)
	tagttcaccatctttcgggtc (R)	Belshaw et al. (2001)



Biosystems) using the BigDye Terminator v1.1 chemistry (Applied Biosystems) at the LSU Genomics Facility. DNA assembly and sequence editing were conducted using Geneious Prime 2021.2 (<https://www.geneious.com>). Edited sequences of 16S and 28S were aligned using MUltiple Sequence Comparison (MUSCLE) (Edgar 2004) on the website of EMBL's European Bioinformatics Institute (<https://www.ebi.ac.uk/Tools/msa/muscle/>) (Madeira et al. 2019) with a default setting. Genetic distances were estimated using MEGA11 (Tamura et al. 2021). The estimates were performed with a Kimura-2-parameter model (K2P) with pairwise deletion for the gaps/missing data treatment.

## Phylogenetic analyses

### Morphological data

Thirty-nine morphological characters for ten *Neocardiochiles* species were selected and included in the phylogenetic analysis (Table 2). Ten characters (as indicated in the table) were based on or modified from Dangerfield et al. (1999). Additionally, 29 new informative characters were discovered and included in the analysis. Characters were coded from females except for *N. whitfieldi* (Mercado, 2003), comb. nov., which is known only from a male specimen. Among 39 characters, 32 characters were coded as binary states and seven were coded as multistate characters (Table 2). To reduce errors in the parsimony analysis, continuous characters were coded as discrete binary or multistates. The character matrix for a phylogenetic analysis was prepared using Mesquite version 3.70 (Maddison and Maddison 2021). In the matrix '?' indicates character states that were not coded because those characters were absent on specimens (Suppl. material 4). Using this matrix, a maximum parsimony (MP) analysis was performed using PAUP\* (Swofford 2021). Heuristic searches were conducted via multiple TBR + TBR hold. All character states were unweighted and unordered. The tree was rooted using *Protomicroplitis calliptera* (Say, 1836) (Braconidae: Microgastrinae) as the outgroup. A list of apomorphic characters was produced using PAUP\* and mapped on the MP phylogeny using Adobe Acrobat Pro DC (Adobe Systems, Inc) (Fig. 1).

### Molecular data

Maximum likelihood analysis (ML) was conducted using MEGA11 (Tamura et al. 2021). For 16S, the analysis was conducted using the General Time Reversible (GTR) model (Nei and Kumar 2000). Bootstrapping was not conducted because only three species were included in the analysis. For 28S, the ML was performed using the Hasegawa-Kishino-Yano model (HKY) with 1,000 bootstrap replicates (Hasegawa et al. 1985). The substitution models were selected using ModelTest-NG (Darriba et al. 2020) on raxmlGUI 2.0 (Edler et al. 2021). All phylogenetic trees were rooted with *Protomicroplitis calliptera* as outgroup (GenBank Accession Numbers: [ON023818.1](#) (16S) and [ON040756.1](#) (28S)) and edited via MEGA11 and Adobe Acrobat Pro DC (Adobe Systems, Inc).



## Results and discussion

### Generic relationships and character discussion (Fig. 1)

The Maximum Parsimony (MP) phylogenetic analysis based on morphological characters was conducted due to the issues with *Heteropteron* and *Wesmaelella* specimens mentioned above, and the limited molecular data obtained from only a few specimens of *Neocardiochiles*. In the MP consensus tree, *Protomicroplitis* was set as outgroup, and

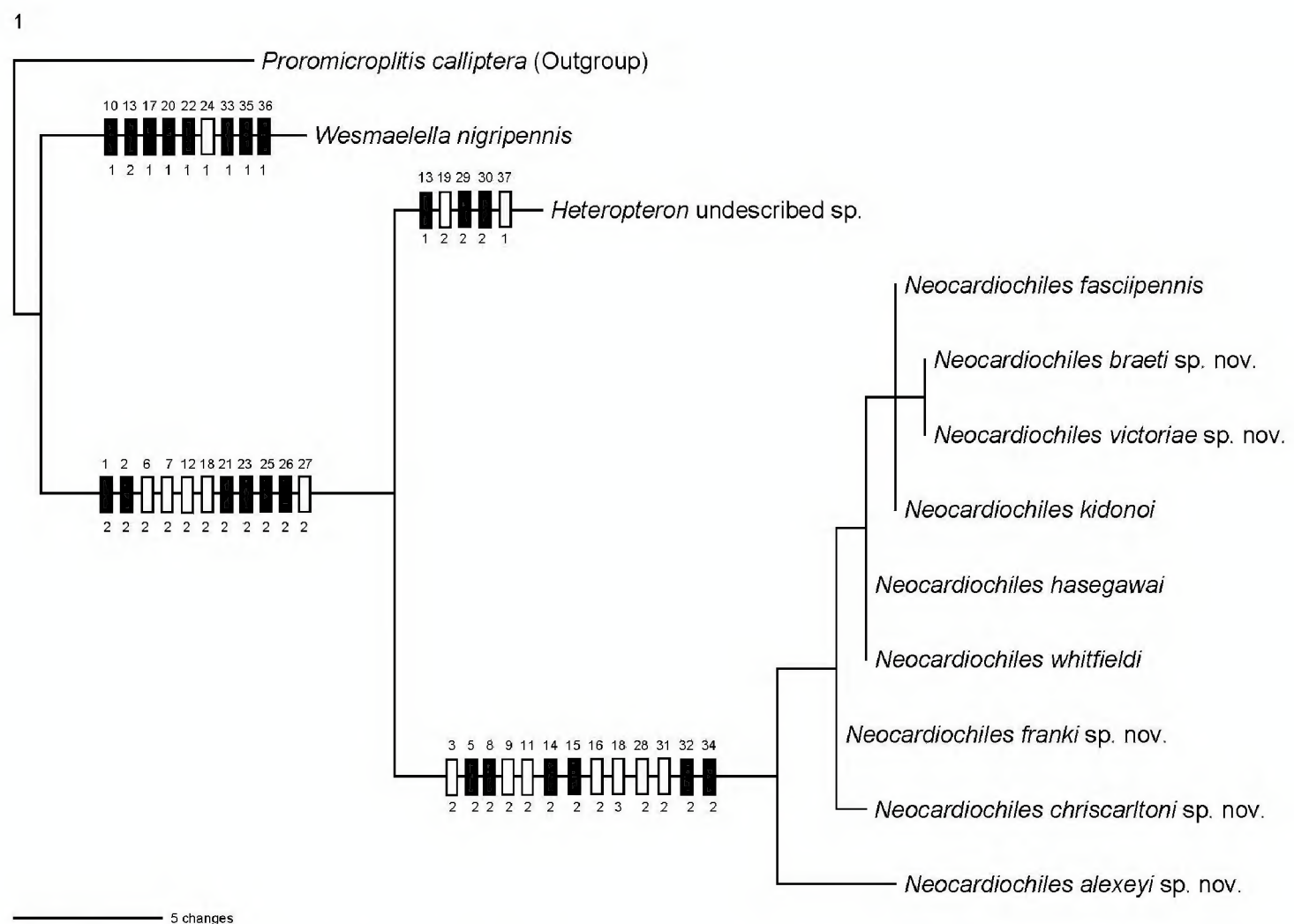
**Table 2.** List of characters and character states used in the data matrix.

Number	Characters	Character states
1	Y-shaped suture on frons	absent = 1; present = 2
2	POD	broad = 1; narrow = 2
3	Malar space width	as long as basal width of mandible = 1; shorter than basal width of mandible = 2
4*	Mouthparts length	short = 1; elongate = 2
5	3 <sup>rd</sup> maxillary palpi shape	moderately swollen apically in lateral view = 1; strongly swollen apically in lateral view = 2
6	Scutellar sulcus shape	transversely straight = 1; transversely curved = 2
7	Scutellar sulcus depth	shallow and evenly impressed = 1; medially shallow, laterally deep = 2
8	Scutellum length	longer than basal width of scutellum = 1; shorter than basal width of scutellum = 2
9	Metascutellum length	long = 1; short = 2
10	Posterior margin of axilla	meeting lateral margin of scutellum with narrow angle = 1; meeting lateral margin of scutellum with broad angle = 2
11	Pronotal carina	absent = 1; present = 2
12	Episternal scrobe	apparent = 1; weakly impressed = 2
13	Ventral margin of metapleuron	without carinate margin = 1; anteriorly carinate = 2; entirely carinate = 3
14*	Median longitudinal furrow on propodeum	absent = 1; present = 2
15	Curved submedial carina on propodeum	absent = 1; present posteriorly = 2
16	Lateral margin of propodeum	absent = 1; carinate = 2
17*	Fore wing 1r	present = 1; absent = 2
18	Fore wing 2RS shape	angled = 1; basally weakly curved = 2; straight = 3
19*	Fore wing 3RSb	broken basally = 1; evenly present = 2
20	Fore wing (RS+M)b length	shorter than m-cu = 1; longer than m-cu = 2
21	Fore wing (RS+M)b angle	meeting 2M with $\sim 140^\circ$ = 1; meeting 2M with $180^\circ$ = 2
22	Fore wing RS2	present as basal stump = 1; absent = 2
23	Fore wing r-m length	$0.5 \times$ longer than height of second submarginal cell = 1; $0.3 \times$ longer than height of second submarginal cell = 2; as long as height of second submarginal cell = 3
24	Fore wing 1cu-a origin	arising from middle of 1CU = 1; arising from basal fourth of 1CU = 2
25*	Fore wing 1a	absent = 1; present = 2
26	Hind wing M+CU length	slightly longer than 1M = 1; shorter than 1M = 2; as long as 1M = 3
27	Hind wing cu-a length	as long as 1M = 1; shorter than 1M = 2
28*	Hind wing 2-1A length	reaching at basal half = 1; not reaching at basal half = 2
29	Second tarsomere of fore leg length	shorter than fifth tarsomere = 1; as long as fifth tarsomere = 2
30	Second tarsomere of middle leg length	shorter than combined length of third and fourth tarsomeres = 1; longer than combined length of third and fourth tarsomeres = 2
31	Basal spur on hind tibia length	$\sim 0.33 \times$ longer than hind basitarsus = 1; $\geq 0.40 \times$ longer than hind basitarsus = 2
32*	Tarsal claws	Simple = 1; pectinate = 2
33*	T1 ratio	$\leq 1.70 \times$ = 1; $\geq 2.0 \times$ = 2
34	First laterotergite	weakly curved posteriorly = 1 strongly curved posteriorly = 2
35	Spiracle of first laterotergite	touching dorsal margin of first laterotergite = 1; located near median = 2
36	Spiracle of second laterotergite	close to anterior margin, but not touching = 1; located near median = 2
37*	Ovipositor sheath length	longer than hind femur = 1; shorter than hind femur; unknown = ?
38	Ovipositor sheath shape	ventro-apically round = 1; ventro-apically pointed = 2; unknown = ?
39*	Median longitudinal fold on hypopygium	absent = 1; present = 2; unknown = ?

\* Characters are based on or modified from Dangerfield et al. (1999)



all the three ingroup genera are recovered as monophyletic groups. Based on the results, we resurrect *Neocardiochiles* to the generic level. A clade including *Wesmaelella* represented by *Wesmaelella nigripennis* (Szépligeti, 1902) was supported by eight synapomorphies (10-1; 13-2; 17-1; 20-1; 22-1; 33-1; 35-1; 36-1) and one homoplastic character (24-1) and recovered as the most plesiotypic member. 1r on fore wing (17-1) and RS2 (22-1) are easily observable characters to distinguish *Wesmaelella* from the members of *Heteropteron* and *Neocardiochiles*. A clade containing *Heteropteron* and *Neocardiochiles* were supported by six synapomorphies (1-2; 2-2; 21-2; 23-2; 25-2; 26-2) and five homoplastic characters (6-2 7-2 12-2 18-2 27-2). Three synapomorphies (13-1; 29-2; 30-2) and two homoplastic characters (19-2; 37-1) supported a clade with *Heteropteron* represented by the undescribed species. All the three unambiguous synapomorphies along with the ovipositor character (37-1; note: the undescribed species of *Heteropteron* possess a distinctively longer and more sinuate ovipositor than the members of *Wesmaelella* and *Neocardiochiles*) are easily visible diagnostic characters to identify *Heteropteron*. A clade including nine species of *Neocardiochiles* was supported by six synapomorphies (5-2; 8-2; 14-2; 15-2; 32-2; 34-2) and seven homoplastic characters (3-2; 9-2; 11-2; 16-2; 18-3; 28-2; 31-2) (Fig.1). Among six unambiguous synapomorphies of *Neocardiochiles*, the presence of a median longitudinal furrow



**Figure 1.** Maximum Parsimony (MP) phylogeny based on morphological data indicating the relationships of *Heteropteron*, *Neocardiochiles* and *Wesmaelella*. *Protomicroplitis calliptera* (Hymenoptera: Braconidae: Microgastrinae) is included as the outgroup. Synapomorphies are mapped on the phylogeny. Black bars indicate non-homoplastic characters, and white bars represent homoplastic characters. Characters are listed above bars, and character states are indicated below bars.



on the propodeum (14-2) and pectinate claws (32-2) are easily observable diagnostic characters to distinguish the *Neocardiochiles* members from members of *Wesmaelella* and *Heteropteron*. Regarding species relationships displayed by the phylogeny (Fig. 1), some *Neocardiochiles* species were not clearly delimited because we excluded characters only informative at the species-level due to the main purpose of the phylogeny (confirming the genus-level relationships). However, species of *Neocardiochiles* can be easily delimited by additional morphological characters and molecular data included below.

## Molecular data

We did not attempt to obtain molecular data from *Heteropteron* and *Wesmaelella* specimens because they were collected in the early 1900s and/or were type specimens. *Neocardiochiles* specimens collected from late 1990s to early 2010s were used to obtain molecular data. Among nine species of *Neocardiochiles*, 16S sequences of two species, *N. chriscarltoni* sp. nov. and *N. victoriae* sp. nov., and 28S sequences of three species, *N. alexeyi* sp. nov., *N. hasegawai* comb. nov., and *N. victoriae* sp. nov., were obtained. Unfortunately, attempts to obtain DNA sequences from the other five species of *Neocardiochiles* failed. In the genetic distance analyses and maximum likelihood analyses, ~465 bp of 16S sequences and ~420 bp of 28S sequences were utilized as the final dataset, respectively. The length of 28S sequences used in the analysis was shorter than our target length because only the forward strand of *N. hasegawai* was successfully obtained. Interspecific genetic distance between *N. chriscarltoni* sp. nov. and *N. victoriae* sp. nov. was 12.1% for 16S (Table 3). For 28S sequences, the distances ranged from 1.3% to 1.5% (Table 4). As shown in the results, 28S interspecific genetic distances between species were much lower than 16S, indicating confirmation of species boundaries based on 28S sequences would be more difficult. 16S sequences exhibited high interspecific genetic distances but mitochondrial cytochrome *c* oxidase subunit I (COI) barcodes obtained using universally known markers (Folmer et al. 1994; Hebert et al. 2004) may be more useful to delimit *Neocardiochiles* species than 16S as confirmed in many other braconid studies (Smith et al. 2008; Smith et al. 2012; Fernandez-Triana et al. 2014; Kang et al. 2017; Fernandez-Triana et al. 2019; Meierotto et al. 2019; Fagan-Jeffries and Austin 2020; Sharkey et al. 2021a, b; Slater-Baker et al. 2022). Two single gene trees (See Suppl. material 1: Maximum likelihood (ML) phylogeny based on 16S data and Suppl. material 2: Maximum likelihood (ML) phylogeny based on 28S data) were constructed, and the ML phylogeny based on 28S (See Suppl. material 2: Maximum likelihood (ML) phylogeny based on 28S data) was congruent with the morphology-based phylogeny (Fig. 1) in confirming a relatively early-diverging position for *N. alexeyi* despite the small number of sequenced taxa (Suppl. material 2).

**Table 3.** Estimates of genetic distances between 16S sequences.

	<i>N. chriscarltoni</i> sp. nov.	<i>N. victoriae</i> sp. nov.	<i>P. calliptera</i> (outgroup)
<i>N. chriscarltoni</i> sp. nov.	0		
<i>N. victoriae</i> sp. nov.	0.121		
<i>P. calliptera</i> (outgroup)	0.101	0.116	0



**Table 4.** Estimates of genetic distances between 28S sequences.

	<i>N. alexeyi</i> sp. nov.	<i>N. hasegawai</i>	<i>N. victoriae</i> sp. nov.	<i>P. calliptera</i> (outgroup)
<i>N. alexeyi</i> sp. nov.	0			
<i>N. hasegawai</i>	0.013			
<i>N. victoriae</i> sp. nov.	0.015	0.013		
<i>P. calliptera</i> (outgroup)	0.255	0.258	0.262	0

Taxonomy

*Neocardiochiles* Szépligeti, 1908

*Heteropteron* Brullé, 1846, synonymized by Dangerfield, Austin, and Whitfield (1999) and confirmed by Mercado and Wharton (2003), Papp (2014), Dabek et al. (2020). Type species: *Heteropteron macula* Brullé, 1846, designated by Viereck (1914) *Heteropteron whitfieldi* Mercado, 2003 to *Neocardiochiles whitfieldi* (Mercado, 2003); *Heteropteron kidonoi* Dabek & Whitfield, 2020 to *Neocardiochiles kidonoi* (Dabek & Whitfield, 2020); *Heteropteron hasegawai* Dabek & Whitfield, 2020 to *Neocardiochiles hasegawai* (Dabek & Whitfield, 2020). New combinations.

**Type species.** *Neocardiochiles fasciipennis* Szépligeti, 1908.

**Diagnosis.** *Neocardiochiles* is most similar to the genera *Heteropteron* and *Wesmaelella* and shares the following characters: eyes without setae; median areola on propodeum absent; notauli weakly impressed and posteriorly absent; scutellar sulcus without any crenula. However, members of *Neocardiochiles* differ from *Heteropteron* and *Wesmaelella* by possessing pectinate claws; propodeum with median longitudinal furrow (Figs 2E, 3E, 4E, 6E, 7D, 8E), posterior submarginal carinae, and carinate lateral margin (Figs 2E, 3E, 4E, 6E, 7D, 8E); hypopygium with median longitudinal fold (Figs 3D, 4D, 6E, 8D).

**Description.** Body 6.5–11 mm. **Head:** Antenna 34–40-segmented. Face width 1.36–1.73 × longer than its height. Interantennal space with well-developed median carina. Width of anterior ocellus 0.96–1.15 × longer than POL. Eyes bulged and without interommatidial setae (Figs 2D, 3C, 4C, 6C, 7C, 8C); median width of eye about 0.90–1.32 × longer than the median width of gena in lateral view. Gena extended ventroposteriorly into sharp prominence. Clypeus 1.64–2.61 × longer than its height; clypeal tubercles absent. Mandible bidentate. Maxillary palpus six-segmented. Labial palpus four-segmented. Galea short. Glossa short. Occipital carina absent. **Mesosoma:** Notauli weakly impressed and absent posteriorly (Figs 2C, 3B, 4B, 6B, 7B, 8B). Scutellar sulcus weakly impressed except for *Neocardiochiles alexeyi* sp. nov., without crenula. Postscutellar depression absent. Pronotum entirely or mostly smooth with ventral longitudinal carina. Mesopleuron mostly smooth; posterior margin crenulate; precoxal sulcus absent (Fig. 5B). Metapleuron mostly smooth. Propodeum 0.39–0.50 × longer than its median width; mostly smooth; with median furrow; curved submarginal longitudinal carina on propodeum present posteriorly; lateral margin of propodeum carinate (Figs 2E, 3E, 4E, 6E, 7D, 8E). **Legs:** Basal spur on mid tibia 0.56–0.71 × longer



than length of basitarsus. Hind tibia without apical cup-like projection; basal spur on hind tibia  $0.48\text{--}0.62 \times$  longer than length of basitarsus. Claws pectinate. **Wings:** Fore wing (RS+M)a vein present; second submarginal cell trapezoid; 1r absent; 3r absent; 3RSb evenly curved. Hind wing 2r-m absent; 2-1A absent. **Metasoma:** T1  $1.06\text{--}2.22 \times$  longer than its posterior width, anterior width  $0.53\text{--}0.83 \times$  longer than posterior width, entirely separated with lateral tergum by suture; Y-shaped suture present. T2 nearly rectangle,  $0.30\text{--}0.49 \times$  longer than its posterior width. Hypopygium with median fold (Figs 3D, 5D, 6E, 8D). Ovipositor sheath nearly straight to slightly downcurved, as long as hind tarsomeres 1-3 combined as long as mesosoma, evenly setose except for base.

**Distribution.** Neotropical region: Costa Rica, Ecuador, French Guiana, Mexico, and Suriname.

**Biology.** The two species for which hosts are known attack pyralid and depressariid caterpillars on *Roupala* (Proteaceae) (Dabek et al. 2020).

**Diversity.** Nine species (Szépligeti 1908; Mercado and Wharton (2003); Dabek et al. 2020; current work).

### Key to species of *Neocardiochiles* of the New World

- 1      A. Hind femur entirely melanic ..... 2
- B. Hind femur bicolored..... 4
- C. Hind femur entirely pale..... 6

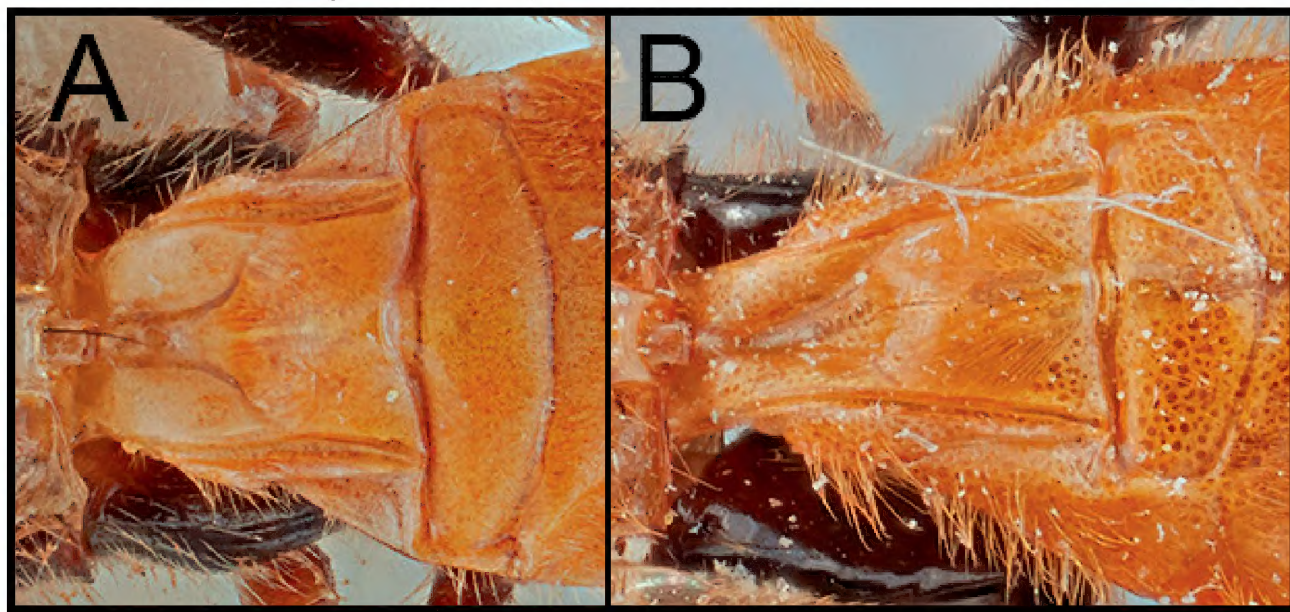


- 2(1)    A. Fore wing entirely infusate..... *N. whitfieldi*
- B. Fore wing two-banded..... 3

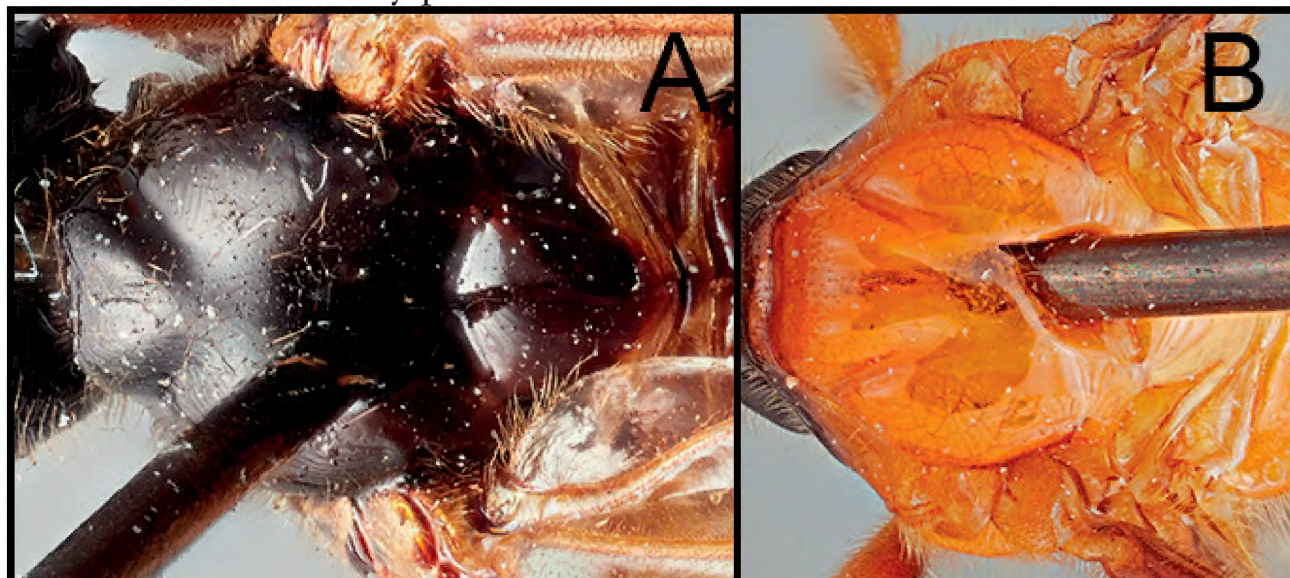




- 3(2) A. T1 ~ 1.06 × longer than its posterior width ..... *N. chriscarltoni* sp. nov.  
 – B. T1 ~ 1.63 × longer than its posterior width ..... *N. braeti* sp. nov.



- 4(1) A. Mesoscutum entirely dark ..... *N. fasciipennis*  
 – B. Mesoscutum entirely pale ..... 5

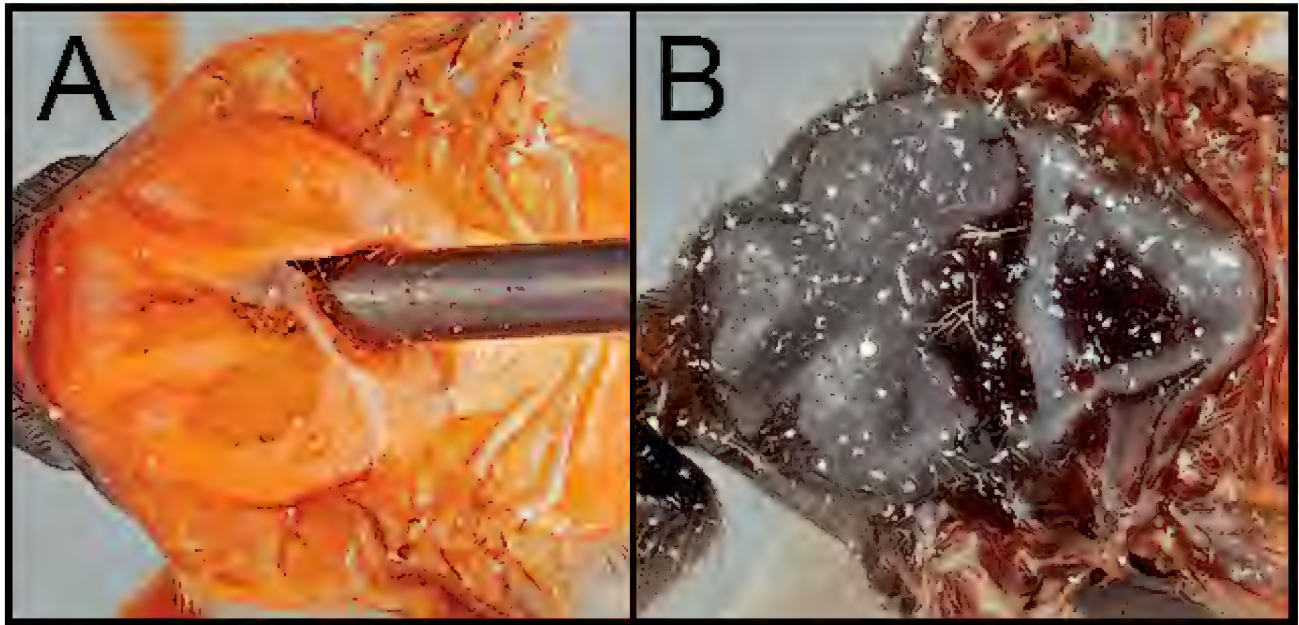


- 5(4) A. Hind tibia mostly pale ..... *N. kidonoi*  
 – B. Hind tibia mostly dark ..... *N. victoriae* sp. nov.

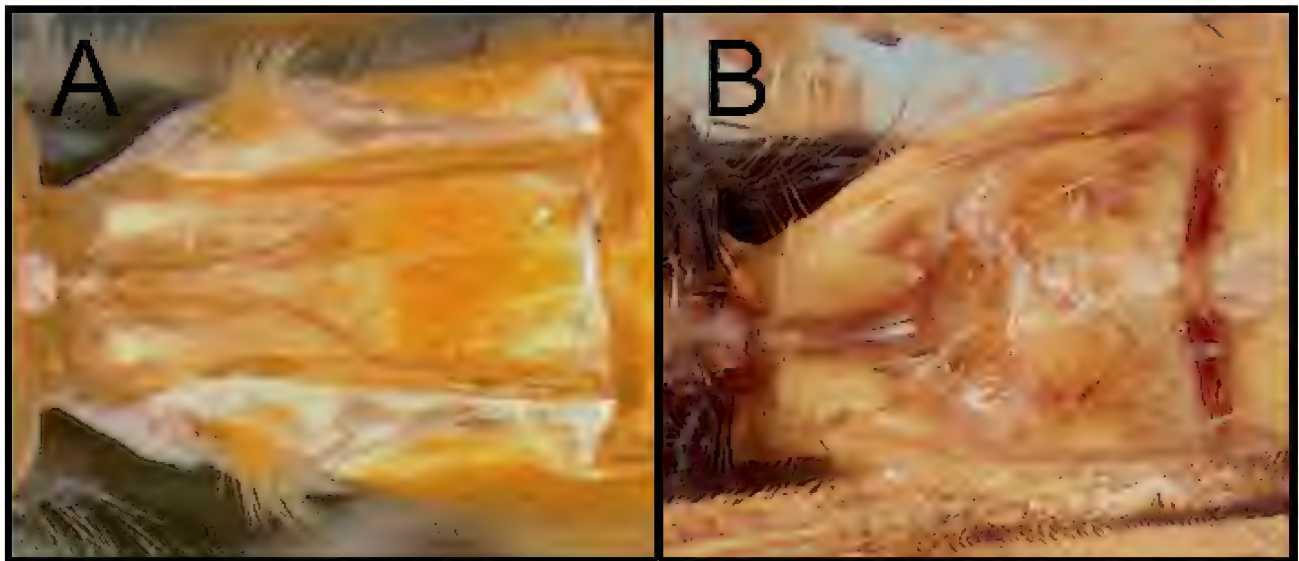




- 6(1) A. Mesoscutum entirely pale ..... *N. hasegawai*  
 – B. Mesoscutum entirely dark ..... 7



- 7(6) A. T1 ~ 2.22 × longer than its posterior width, nearly rectangle .....  
 ..... *N. alexeyi* sp. nov.  
 – B. T1 ~ 1.09 × longer than its posterior width, trapezoid.. *N. franki* sp. nov.



### Interactive key

To use the interactive key to species of *Neocardiochiles* in the New World (Suppl. material 3):

1. Download the zipfile of the ALA version of DELTA Editor (Open DELTA 1.02 beta) (Dallwitz et al. 1999). Extract into the 'C' drive. The extracted folder name will be 'open-delta-1.02-bin'. Java is required to operate DELTA Editor and Intkey.
2. Download the folder ('Neocardiochiles\_DELTA\_Key'), which includes all source files and images of the interactive key, using the following link (<https://drive.google.com/drive/folders/1aLS3vzdK52uMV0702IopDWgXaSlpJQm?usp=sharing>).
3. Extract the zipfile of 'Neocardiochiles\_DELTA\_Key' into the 'open-delta-1.02-bin' folder.



4. In the folder 'Neocardiochiles\_DELTA\_Key', open the file 'Neocardiochiles\_Intkey'.
5. Users can then identify described species of *Neocardiochiles* using this interactive key.

## Species descriptions

***Neocardiochiles alexeyi* Kang, sp. nov.**

<http://zoobank.org/A3A772FE-6C09-4B6A-8CF2-63C9A71E3AD4>

Fig. 2A–E

**Material examined.** *Holotype* ECUADOR • ♀; Yasuni Research Station, Yasuni National Park, Orellana province; 00°40.4'S, 76°23.861'W; 18–24.vii.2008; A. Tishechkin; AT 853MS-2; H16634. Will be deposited in CNC.

**Diagnosis.** *Neocardiochiles alexeyi* sp. nov. can be distinguished from other members of the *Neocardiochiles* by the combination of the following characters: antenna 34-segmented and with pale apex (Fig. 2A); glossa  $\sim 2.0 \times$  longer than height of clypeus (Fig. 2D); scutellar sulcus moderately impressed (Fig. 2B); mesoscutum entirely dark; hind femur entirely pale; anterior width of median furrow of propodeum  $\sim 0.83 \times$  longer than maximum width (Fig. 2E); T1  $\sim 2.22 \times$  longer than its posterior width; lateral sutures of T1 nearly parallel (Fig. 2E); T2  $\sim 0.49 \times$  longer than its posterior width (Fig. 2E); ovipositor sheath  $\sim 0.62 \times$  longer than length of hind tibia (Fig. 2A).

**Molecular data.** 28S sequences (GenBank accession number: [ON040823.1](https://www.ncbi.nlm.nih.gov/nuclot/ON040823.1));

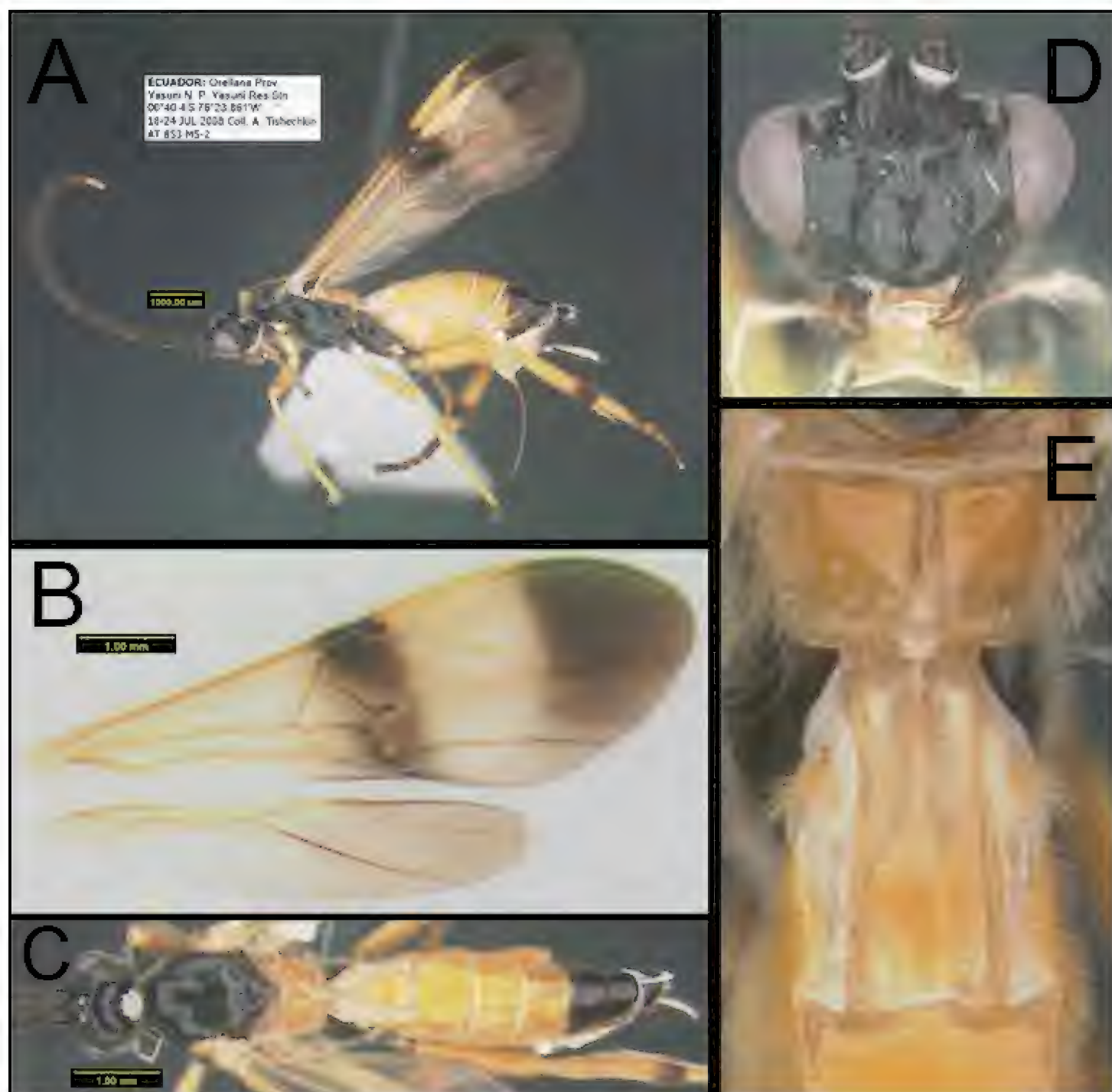
ATGAGGAGATTCACTGTTAGCATTACTAGTATTAATGCAAATTATGAT  
ATGATTATATGATCCTTGTGGTCACAATTATTATACTTATTTGTATTATTT  
TATTGGTTTTTGTTCAGCATGCACTTCTCCTCTAGTAGAACGTCGCGACCC  
GTTGAATGTTTATTTATGAGCCACATGGTAGTCTTATGTATTTTATACGC  
AAGACCAGTGAATTTCTAATAAACTATTTGACGGTATCTAAAATGGTATT  
GAGCCGCAAATTTTTTTTGTGCGTTAGATTTATTACAAGCTAGACTTACTTT  
AAGCAGTACGAATTTTATGTCGTCGTTTAACTAGTCTGCTGTTAGTGA  
TAATATCTTTAACTGGCTTAATTTTACCGGTCAGCGATGCTACTGCTTTG  
GGTACTTACAGGACCCGTCTTG.

**Description.** Body  $\sim 7.04$  mm. **Head:** Antenna 34-segmented. Face width  $\sim 1.36 \times$  wider than high (1.01:0.74). Width of anterior ocellus as long as POL (0.10:0.10). Eyes bulged and without interommatidial setae; median width of eye about  $\sim 1.23 \times$  longer than the median width of gena in lateral view (0.37:0.30). Clypeus  $2.20 \times$  longer than its height (0.55:0.25). Galea  $1.64 \times$  longer than height of clypeus (0.41:0.25), relatively longer than other members of the genus. Glossa  $1.96 \times$  longer than height of clypeus (0.49:0.25). Occipital carina absent. **Mesosoma:** Notauli weakly impressed at anterior two thirds and absent posteriorly. Scutellar sulcus straight, moderately impressed, medially shallow, laterally relatively deep, without crenula. Pronotum mostly smooth, with ventral longitudinal carina. Metapleuron mostly smooth. Propodeum  $\sim 0.48 \times$  longer than its median width (0.40:0.83); median longitudinal furrow present, nearly rectangle, anteriorly opened posteriorly



closed by nucha, anterior width  $\sim 0.83 \times$  longer than maximum width (0.05:0.06). **Legs:** Basal spur on mid tibia  $\sim 0.60 \times$  longer than length of basitarsus (0.38:0.63). Basal spur on hind tibia  $\sim 0.54 \times$  longer than length of basitarsus (0.60:1.11). **Wings:** Fore wing (RS+M)a present; second submarginal cell trapezoid,  $\sim 2.81 \times$  longer than height (1.32:0.47); 1r absent; 3r absent; 3RSb evenly curved. Hind wing 2r-m absent; 2-1A absent. **Metasoma:** T1  $\sim 2.22 \times$  longer than its posterior width (0.91:0.41), anterior width  $\sim 0.83 \times$  longer than posterior width (0.34:0.41), dorsally nearly rectangle; Y-shaped suture present. T2 nearly rectangle,  $\sim 0.49 \times$  longer than its posterior width (0.35:0.71), with straight posterior margin. Hypopygium with median fold. Ovipositor sheath nearly straight,  $\sim 0.62 \times$  longer than length of hind tibia (1.31:2.11), evenly setose except for base.

**Color.** Body mostly yellowish pale. The following areas dark: basal antenna (mostly), head, mandible, mesonotum, pronotum, mesopleuron, metapleuron, middle coxa,



**Figure 2.** *Neocardiochiles alexeyi* sp. nov. **A** lateral habitus **B** wings **C** dorsal habitus **D** dorsal anterior head **E** propodeum and T1–T2.



hind coxa, apical hind tibia, apical hind tarsi, apical metasoma. Fore wing with two bands; stigma mostly pale.

**Etymology.** Named in honor of Dr. Alexey K. Tishechkin, staff in the Plant Pest Diagnostics Branch, California Department of Food & Agriculture and a former member of the Louisiana State Arthropod Museum at LSU AgCenter, who collected the specimen in Ecuador.

**Male.** Unknown.

**Host.** Unknown.

**Distribution.** *Neocardiochiles alexeyi* sp. nov. is known from only one female specimen collected from Yasuni Biological Station, Orellana province, Ecuador.

***Neocardiochiles braeti* Kang, sp. nov.**

<http://zoobank.org/2B598860-7AC1-41A3-831E-5E6EB79F526E>

Fig. 3A–E

**Material examined.** *Holotype* FRENCH GUIANA • ♀; Roura, Montagne des chevaux; 22.xi.2008; S.E.A.G.; 2008–2009. Will be deposited in RBINS.

**Diagnosis.** *Neocardiochiles braeti* sp. nov. can be distinguished from other *Neocardiochiles* species by the combination of the following characters: antenna  $\geq 37$ -segmented; scutellar sulcus weakly impressed (Fig. 3B); fore wing with two bands; hind femur entirely melanic; anterior width of median furrow of propodeum  $\sim 0.33 \times$  longer than maximum width (Fig. 3E); T1  $\sim 1.63 \times$  longer than its posterior width; T2  $\sim 0.42 \times$  longer than its posterior width (Fig. 3E); ovipositor sheath as long as hind tibia (Figs 3A, 3B, 3D).

**Description.** Body  $\sim 8.67$  mm. **Head:** Antenna  $\geq 37$ -segmented, apically broken. Face width  $\sim 1.46 \times$  longer than its height (1.02:0.70) Width of anterior ocellus  $\sim 0.96 \times$  longer than POL. Eyes bulged and without interommatidial setae; median width of eye about  $\sim 0.90 \times$  longer than median width of gena in lateral view (0.44:0.49). Clypeus  $\sim 2.61 \times$  longer than its height (0.60:0.23). Malar space  $0.84 \times$  longer than basal width of mandible. **Mesosoma:** Notauli anteriorly weakly impressed and absent posteriorly. Scutellar sulcus weakly impressed, medially shallow, laterally relatively deep, without crenula. Pronotum entirely smooth. Propodeum  $\sim 0.44 \times$  longer than its median width (0.57:1.30); median longitudinal furrow present, elongate isosceles trapezoid, anteriorly opened posteriorly closed by nucha, anterior width  $\sim 0.33 \times$  longer than maximum width (0.04:0.12). **Legs:** Basal spur on mid tibia  $\sim 0.64 \times$  longer than length of basitarsus (0.47:0.74). Basal spur on hind tibia  $\sim 0.50 \times$  longer than length of basitarsus (0.68:1.37). **Wings:** Fore wing (RS+M)a vein present; second submarginal cell trapezoid,  $\sim 3.01 \times$  longer than height (1.66:0.55); 1r absent; 3r absent; 3RSb evenly curved; stigma about  $\sim 4.42 \times$  longer than wide medially (2.08:0.47). Hind wing 2r-m absent; 2–1A absent. **Metasoma:** T1  $\sim 1.63 \times$  longer than its posterior width (1.19:0.73), anterior width  $\sim 0.63 \times$  longer than posterior width (0.46:0.73), dorsally isosceles trapezoid; Y-shaped suture partially developed anteriorly, absent posteriorly.



T2 nearly trapezoid,  $\sim 0.42 \times$  longer than its posterior width (0.44:1.06), with curved posterior margin. Hypopygium with median fold. Ovipositor sheath slightly downcurved,  $\sim 1.03 \times$  longer than length of hind tibia (2.92:2.81), evenly setose except for base.

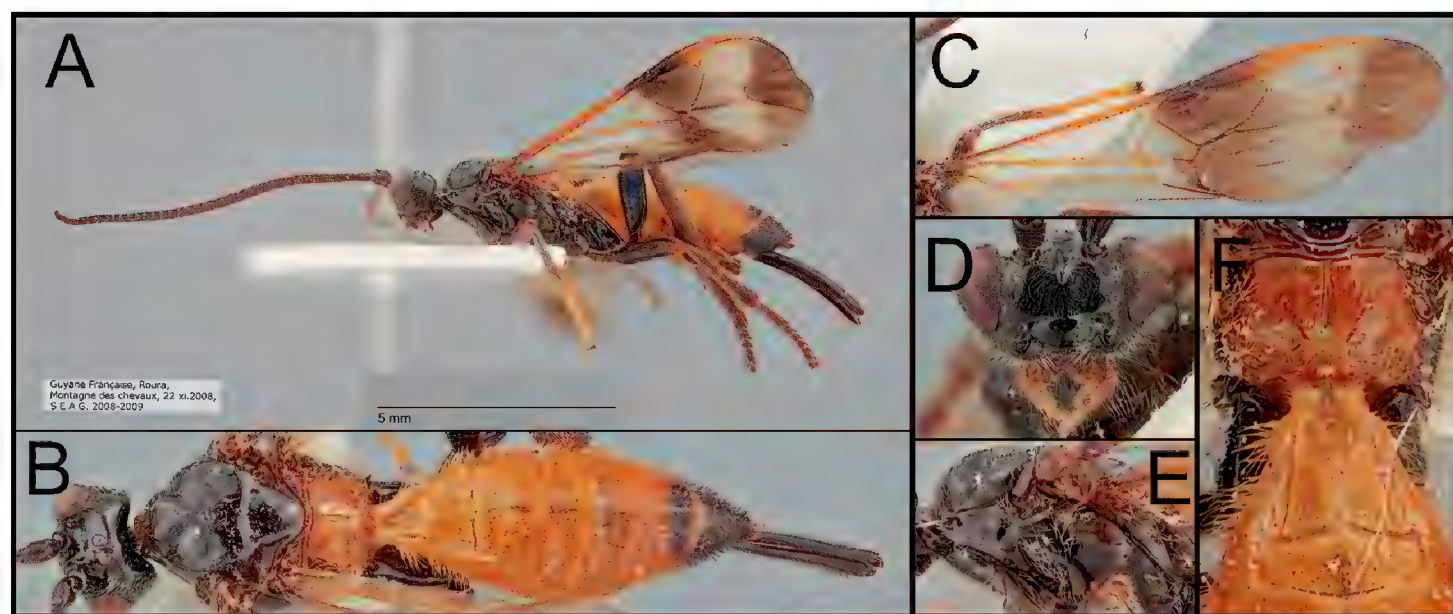
**Color.** Head and mesosoma mostly black, metasoma mostly orange and black apically. Legs black or dark brown except for fore tarsus. Fore wing with two bands; stigma pale at apical two thirds.

**Etymology.** Named in honor of Mr Yves Braet who provided specimens collected in French Guiana for this study.

**Male.** Unknown.

**Host.** Unknown.

**Distribution.** *Neocardiochiles braeti* sp. nov. is known only from one female specimen collected from Roura, Montagne des chevaux, French Guiana.



**Figure 3.** *Neocardiochiles braeti* sp. nov. **A** lateral habitus **B** dorsal habitus **C** fore wing **D** anterior head **E** lateral mesosoma **F** dorsal propodeum and T1.

***Neocardiochiles chriscarltoni* Kang, sp. nov.**

<http://zoobank.org/294D2CD0-0762-47EA-BB20-4115F21D9F0C>

Figs 4A–E, 5A–B

**Material examined.** *Holotype* FRENCH GUIANA • ♀; Roura, Montagne des chevaux; ix.2009; S.E.A.G.; 2008–2009, Piège Malaise. Will be deposited in RBINS.

**Diagnosis.** *Neocardiochiles chriscarltoni* sp. nov. is most similar to *N. braeti* sp. nov. However, *N. chriscarltoni* sp. nov. can be distinguished from other *Neocardiochiles* species by the combination of the following characters: antenna 38-segmented and with pale apex (Fig. 5A); scutellar sulcus weakly impressed (Fig. 4B); fore wing with two bands; hind femur entirely melanic; anterior width of median furrow of propodeum  $\sim 0.11 \times$  longer than maximum width (Fig. 4E); T1 as long as its width (Fig. 4E); T2  $\sim 0.30 \times$  longer than its posterior width (Fig. 4E); ovipositor sheath  $\sim 0.48 \times$  longer than length of hind tibia (Fig. 4A, D).



**Molecular data.** 16S sequences (GenBank accession number: [ON059709.1](https://www.ncbi.nlm.nih.gov/nuotide/ON059709.1));

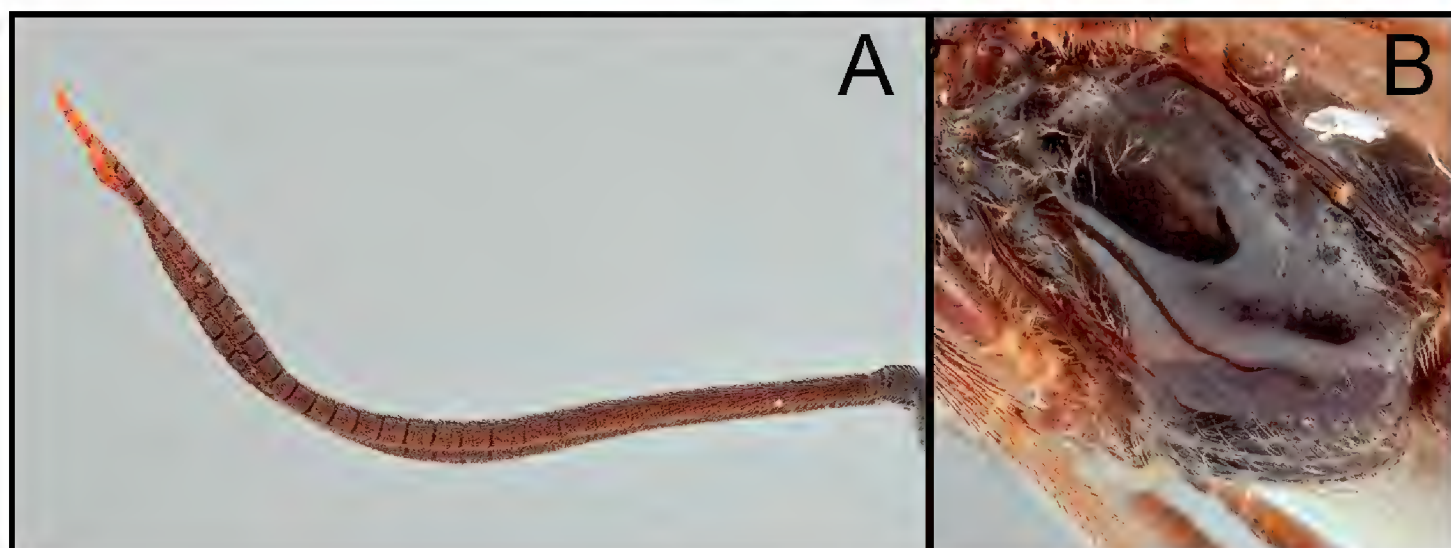
CACCTGTTTATCAAAAACATGTCTTTTTTGAAAATAATTTAAAGTC-  
CAATCTGCTCAATGATTAATTAATTTAATAGCTGCAATATTTATAATTG-  
TACTAAGGTAGCATAATCATTAGTTTATTAATTGTAAACTTGTATGAAC-  
GATTTGATGAAATAAATACTATTTTCATTTTAAAAAAATAAATTTTTTTT-  
TAAGTTAAAAAACTTAAATAATATTAAGACGAGAAGACCCTTTA-  
GAATTTTATAATAATAATTTATAAAAATTTTTTATATATTTATAAATAAT-  
TATTATTTAATTGGGGTGATTATAAAAATTTAATAAACTTT-  
TATATAAATAAACAATAATTTTTTGAATAAAAATATATTTTTTTT-  
TAAAAAAATAAATTAAATTACCTAAGGGATAACAGCATAATTTTTT-  
TAAAAAGCACAAATTTATAAAAAAGTTTATGACCTCGATGTTGAATTAAGA.

**Description.** Body ~ 7.43 mm. **Head:** Antenna 38-segmented. Face width ~ 1.47 × longer than its height (0.97:0.66). Width of anterior ocellus as long as POL (0.12:0.12). Eyes bulged and without interommatidial setae; median width of eye about ~ 1.32 × longer than the median width of gena in lateral view (0.50:0.38). Clypeus ~ 2.57 × longer than its height (0.59:0.23). **Mesosoma:** Notauli weakly impressed at anterior half and disappeared posteriorly. Scutellar sulcus weakly impressed, medially shallow, laterally relatively deep, without crenula. Pronotum mostly smooth, with ventral longitudinal carina. Propodeum ~ 0.39 × longer than its median width (0.53:1.36); median longitudinal furrow present, elongate isosceles trapezoid, anteriorly opened posteriorly closed by nucha, anterior width ~ 0.11 × longer than maximum width (0.02:0.18). **Legs:** Basal spur on mid tibia ~ 0.56 × longer than length of basitarsus (0.38:0.68). Basal spur on hind tibia ~ 0.48 × longer than length of basitarsus (0.52:1.08). **Wings:** Fore wing (RS+M)a vein present; second submarginal cell trapezoid; 1r absent; 3r absent; 3RSb evenly curved. Hind wing 2r-m absent; 2–1A absent. **Metasoma:** T1 ~ 1.06 × longer than its posterior width (0.82:0.77), anterior width ~ 0.66 × longer than posterior width (0.51:0.77), dorsally nearly rectangular; Y-shaped suture present.



**Figure 4.** *Neocardiochiles chriscarltoni* sp. nov. **A** lateral habitus **B** dorsal habitus **C** anterior head **D** hypopygium and ovipositor sheath **E** dorsal propodeum and T1–T3.





**Figure 5.** *Neocardiochiles chriscarltoni* sp. nov. **A** antennae **B** mesopleuron.

T2 nearly rectangle,  $\sim 0.30 \times$  longer than its posterior width (0.38:1.28), with curved posterior margin. Hypopygium with median fold. Ovipositor sheath nearly straight and posteriorly enlarged,  $\sim 0.48 \times$  longer than length of hind tibia (1.07:2.25), evenly setose except for base.

**Color.** Body mostly melanic; the following areas pale: four apical flagellomeres, basal maxillary and labial palpi, fore tarsi, metanotum (mostly), propodeum, anterior metasoma (mostly). Fore wing with two bands; stigma entirely melanic.

**Etymology.** Named in honor of Dr Christopher E. Carlton, the emeritus professor in the Department of Entomology at LSU AgCenter.

**Male.** Unknown.

**Host.** Unknown.

**Distribution.** *Neocardiochiles chriscarltoni* sp. nov. is known only from one female specimen collected from Roura, Montagne des chevaux, French Guiana.

***Neocardiochiles fasciipennis* Szépligeti, 1908, comb. nov.**

Fig. 6A–E

**Material examined.** *Lectotype* Suriname • ♀; Michaelis.

**Diagnosis.** Morphological characters of *Neocardiochiles fasciipennis* are only known from holotype. The species is distinguished from other members of *Neocardiochiles* by the combination of the following characters: body  $\sim 11$  mm; posterior margin of mesopleuron smooth; basal spur on hind tibia slightly shorter than the half length of hind basitarsus (Fig. 6A); forewing length  $\sim 13$  mm; T1  $\sim 1.35 \times$  longer than apical width (Fig. 6E); ovipositor sheath as long as hind tarsomeres 1–3 combined.

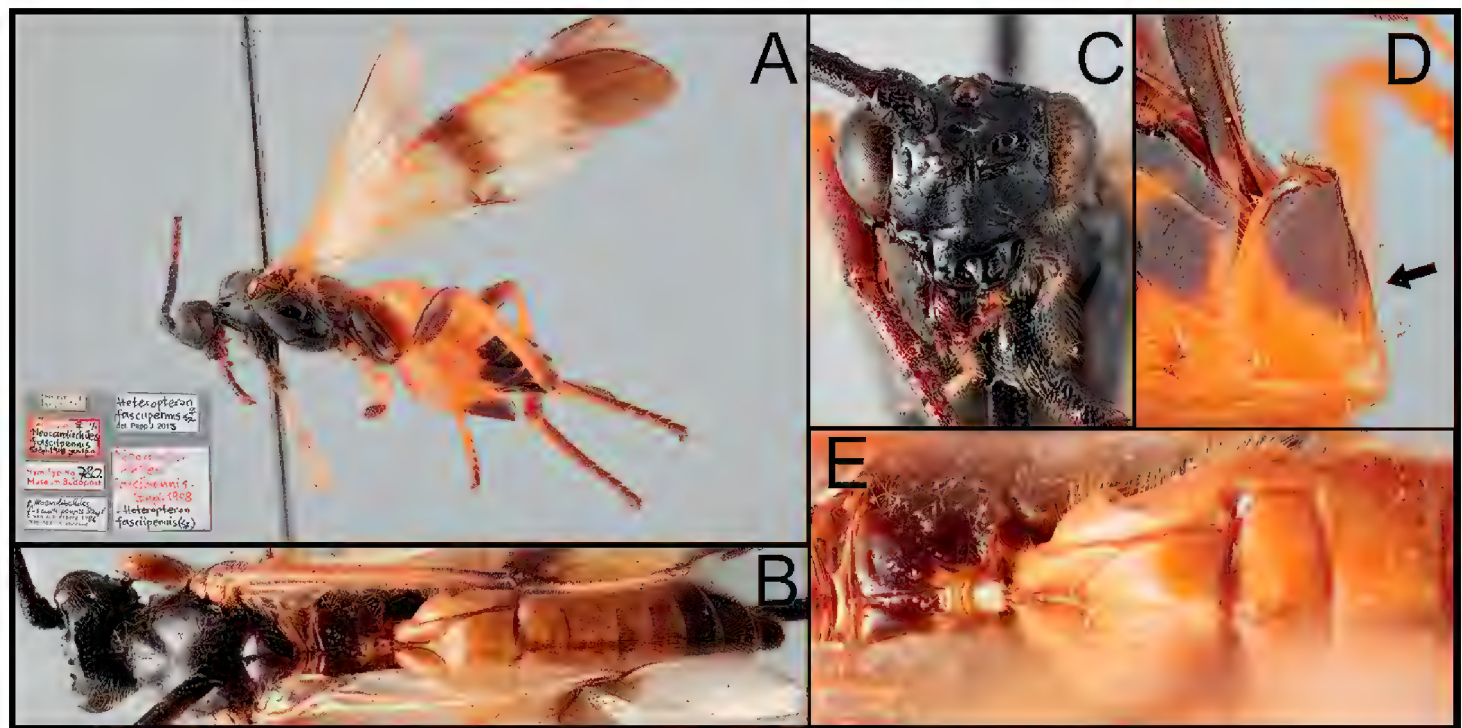
**Description.** See Papp (2014).

**Male.** Unknown.

**Host.** Unknown.

**Distribution.** Suriname.





**Figure 6.** *Neocardiochiles fasciipennis* **A** lateral habitus **B** dorsal habitus **C** anterior head **D** ventro-lateral hypopygium; arrow: median longitudinal fold **E** dorsal propodeum and T1–T3

***Neocardiochiles franki* Kang, sp. nov.**

<http://zoobank.org/475828BF-D48F-4362-A4B9-9FEDE8842873>

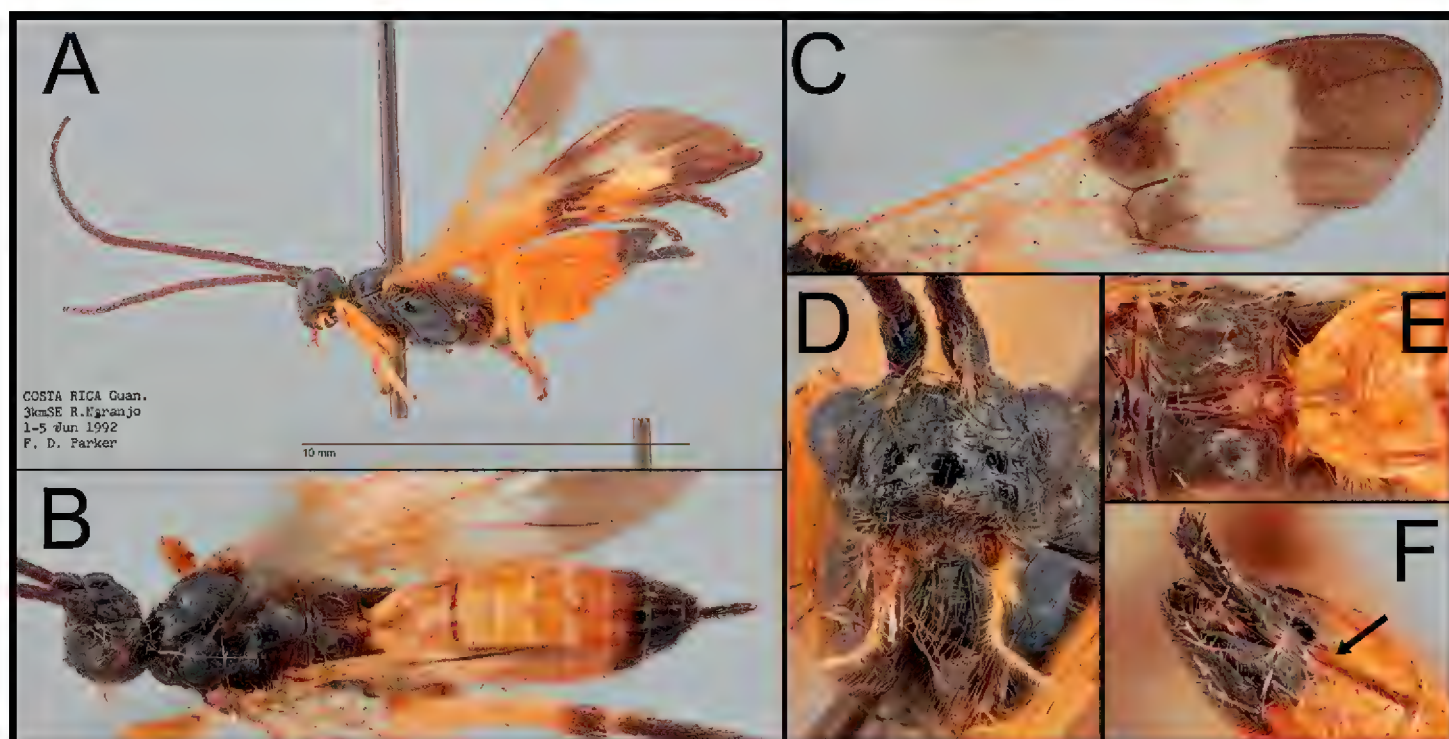
Figs 7A–E

**Material examined.** *Holotype* COSTA RICA • ♀; 3 km SE of Rio Naranjo, Guanacaste; 1–5.vi.1992; F. D. Parker. Will be deposited in UWIM.

**Diagnosis.** *Neocardiochiles franki* sp. nov. is most similar to *N. alexeyi* sp. nov. However, *N. franki* sp. nov. can be distinguished from other *Neocardiochiles* species by the combination of the following characters: antenna  $\geq 37$ -segmented; scutellar sulcus weakly impressed (Fig. 7B) mesoscutum entirely dark; hind femur entirely pale; anterior width of median furrow of propodeum  $\sim 0.18 \times$  longer than maximum width (Fig. 7D); T1 trapezoid and as long as its width (Fig. 7B); T2,  $\sim 0.34 \times$  longer than its posterior width (Fig. 7B); ovipositor sheath  $\sim 0.58 \times$  longer than length of hind tibia (Fig. 7A).

**Description.** Body  $\sim 9.36$  mm. **Head:** Antenna  $\geq 37$ -segmented, apically broken. Face width  $\sim 1.50 \times$  longer than its height (1.11:0.74). Width of anterior ocellus  $\sim 1.11 \times$  longer than POL (0.10:0.09). Eyes bulged and without interommatidial setae; median width of eye about  $\sim 1.32 \times$  longer than median width of gena in lateral view (0.47:0.44). Clypeus  $\sim 2.41 \times$  longer than its height (0.77:0.32). **Mesosoma:** Notauli weakly impressed at anterior half and absent posteriorly. Scutellar sulcus weakly impressed, medially shallow, laterally relatively deep, without crenula. Pronotum mostly smooth, with ventral longitudinal carina. Propodeum  $0.50 \times$  longer than its median width (0.61:1.22); median longitudinal furrow present, elongate isosceles trapezoid, anteriorly opened posteriorly closed by nucha, anterior width  $\sim 0.18 \times$  longer than maximum width (0.03:0.17). **Legs:** Basal spur on mid tibia  $\sim 0.60 \times$  longer than length of basitarsus (0.47:0.78). Basal spur on hind tibia  $\sim 0.62 \times$  longer than length of basitarsus (0.83:1.33). **Wings:** Fore wing (RS+M)a vein present; second submarginal cell trapezoid,  $\sim 2.20 \times$  longer than height (1.81:0.82); 1r





**Figure 7.** *Neocardiochiles franki* sp. nov. **A** lateral habitus **B** dorsal habitus **C** fore wing **D** anterior head **E** dorsal propodeum and T1 **F** hypopygium, arrow: median longitudinal fold.

absent; 3r absent; 3RSb evenly curved. Hind wing 2r-m absent; 2-1A absent. **Metasoma:** T1  $\sim 1.09 \times$  longer than its posterior width (1.20:1.10), anterior width  $\sim 0.53 \times$  longer than posterior width (0.58:1.10), dorsally nearly trapezoid; Y-shaped suture present. T2 rectangle,  $\sim 0.34 \times$  longer than its posterior width (0.54:1.58), with straight posterior margin. Hypopygium with median fold. Ovipositor sheath nearly straight and posteriorly enlarged,  $\sim 0.58 \times$  longer than length of hind tibia (1.82:3.15), evenly setose except for base.

**Color.** Body mostly melanic; the following areas pale: maxillary and labial palpi, fore leg, middle femur; mid tibia; basal middle tarsus, hind femur, basal hind tibia, anterior metasoma (mostly). Fore wing with two bands; stigma mostly pale.

**Etymology.** Named in honor of Dr Frank Parker, the former head of the USDA Bee lab, who collected the specimen.

**Male.** Unknown.

**Host.** Unknown.

**Distribution.** *Neocardiochiles franki* sp. nov. is known only from one female specimen collected from 3 km SE of Rio Naranjo, Guanacaste, Costa Rica.

***Neocardiochiles hasegawai* (Dabek & Whitfield, 2020), comb. nov.**

**Material examined. Non-type specimen** COSTA RICA • ♀; Guanacaste, Area de Conservación Guanacaste, Sector Santa Rosa, Finca Jenny; 10.86333, -85.57443, 205 m; 14.xii.2010 (host caterpillar collection date); 01.i.2011 (host caterpillar pre-pupal date); 17.i.2011 (parasitoid eclosion date); Johan Vargas; DHJPAR0045389; host caterpillar (10-SRNP-15361; *Stenoma cathosiota*) on host plant (*Roupala montana*).

**Diagnosis.** *Neocardiochiles hasegawai* is most similar to *N. alexeyi* sp. nov. and *N. franki* sp. nov., but the members of *N. hasegawai* differ from other *Neocardiochiles*



species by possession of the two following characters: mesoscutum entirely pale; hind femur entirely pale.

**Molecular data.** 28S sequences (GenBank accession number: [ON040755.1](#));

ATGAGGAGATTCACCTGTTAGCATTACTAGTATTAATGCAAATTATGATATGATTATATATGATTCTTGTGGTCACAATTATTATACTTATTTGTATTATTTTATTGGTTTTTGTTCAGCATGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGAATGTTTATTTATGAGTCACATGGTAGTCTTATGTATTTTATACGCAAGACCAGTGAATTTCTAATAAACTGTTTGACGGTATCTAAAATGGTATTGAGCCGCAAATTTTTTTTTTGGCGTTAGATTTATCACAAGCTAGGCTTACTTTAAGCAGTACGAATTTTATGTCGTCGTTTAACTAGTCTGCTGTTAGTGATAATATCTTTAACTGGCTTAATTACCGGTCAGCGATGCTACTGCTTTGGGTACTTACAGGACCCGTCTTG.

**Description.** See Dabek et al. (2020).

**Male.** Body length slightly longer than female (Dabek et al. 2020).

**Host.** Reared from caterpillars of *Carthara abrupta* (Pyralidae) on *Roupala montana* (Proteaceae) (Dabek et al. 2020).

**Distribution.** Costa Rica (ACG).

***Neocardiochiles kidonoi* (Dabek & Whitfield, 2020), comb. nov.**

**Material examined. Non-type specimen** COSTA RICA • ♀; Guanacaste, Area de Conservación Guanacaste, Sector El Hacha, Quebrada Pitahaya; 11.01182, -85.53168; 320 m; 11.ix.2013 (host caterpillar collection date); 14.ix.2013 (host caterpillar pre-pupal date); 02.x.2013 (parasitoid eclosion date); Roster Moraga; DHJPAR0053597; host caterpillar (13-SRNP-22162; *Stenoma cathosiota*) on host plant (*Roupala montana*).

**Diagnosis.** *Neocardiochiles kidonoi* is most similar to *N. victoriae* sp. nov., but members of *N. kidonoi* can be distinguished from other *Neocardiochiles* members by possession of the following characters: mesoscutum entirely pale; hind femur bicolored; hind tibia mostly pale.

**Description.** See Dabek et al. (2020).

**Male.** Body length slightly shorter than female (Dabek et al. 2020).

**Host.** Reared from larvae of *Stenoma cathosiota* (Depressariidae) on *Roupala montana* (Proteaceae) (Dabek et al. 2020).

**Distribution.** Costa Rica (ACG).

***Neocardiochiles victoriae* Kang, sp. nov.**

<http://zoobank.org/432A06CB-A4CC-4773-864E-703BB836B023>

Fig. 8A–E

**Material examined. Holotype** FRENCH GUIANA • ♀; Degrad Laurens, Crique Sapokaï; 95 m; 24.x.–30.x.1998; leg. A.E.I. guyane; P Malaise. Will be deposited in RBINS.



**Diagnosis.** *Neocardiochiles victoriae* sp. nov. is most similar to *N. kidonoi*, but the only known female member of *N. victoriae* differs from other members of *Neocardiochiles* by the following characters: antenna 40-segmented with entirely reddish flagellomeres (Fig. 8A); scutellar sulcus weakly impressed; mesoscutum entirely pale; hind femur bicolored; hind tibia mostly melanic; anterior width of median furrow of propodeum  $\sim 0.19 \times$  longer than maximum width (Fig. 8E); T1  $\sim 1.39 \times$  longer than its posterior width (Fig. 8E); T2  $\sim 0.39 \times$  longer than its posterior width (Fig. 8E); ovipositor sheath as long as hind tibia (Fig. 8A).

**Molecular data.** 28S sequences (GenBank accession number: [ON040754.1](#));

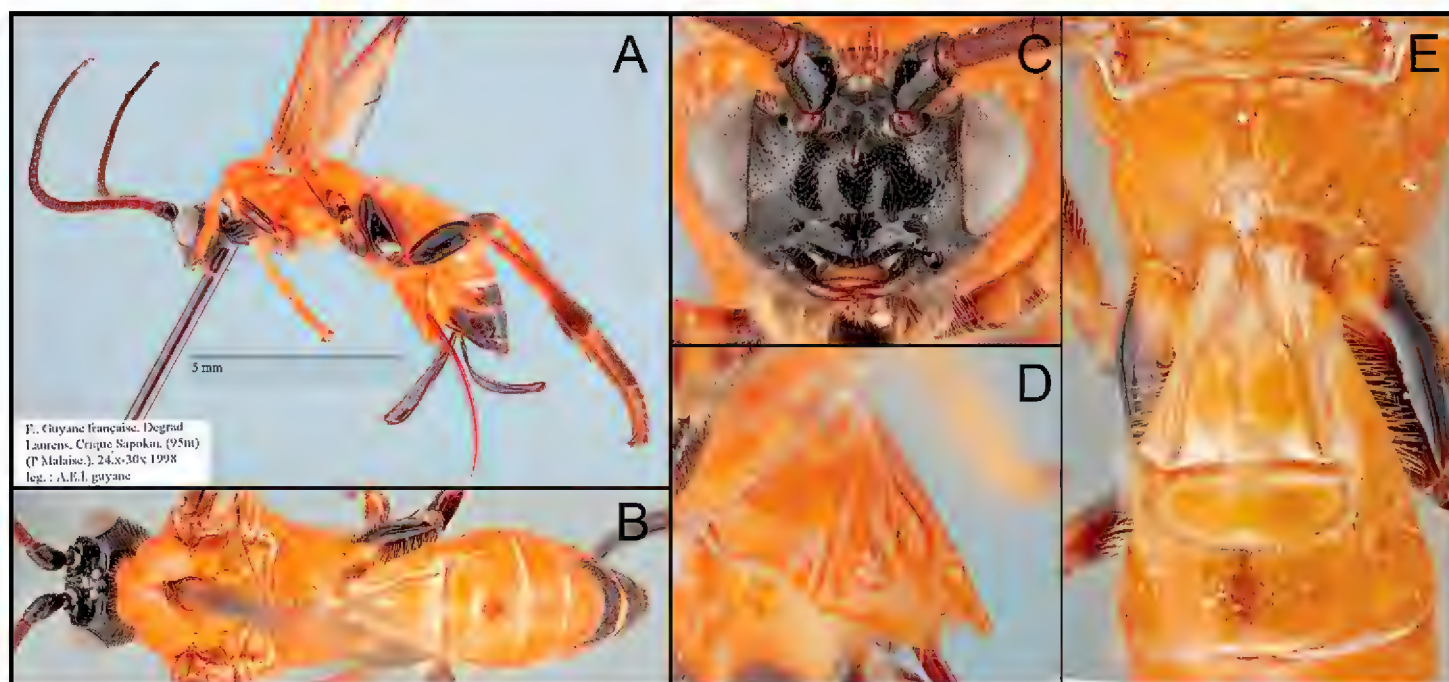
ATGAGGAGATTCACTGTTAGCATTACTAATATTAATGCAAAT-TATGATATCATTATATGATCCTTGTGGTCACAATTATTATACTTATTT-GTATTATTTTATTGGTTTTTGTTCAGCATGCACTTCTCCTCTAGTA-GAACGTCGCGACCCGTTAAATGTTTATTTATGAGTCACATGGTAGTCT-TATGTATTTTATACGCAAGACCAGTGAATTTCTAATAAACTGTTTGACG-GTATCTAAAATGGTATTGAGCCGCAAATTTTTTTTGGCGTTAGATTTAT-TACAAGCTAGGCTTACTTTAAGCAGTACGAATTTTATGTCGTCGTT-TAAACTAGTCTGCTGTTAGTGATAATATCTTTAACTGGCTTAATTACCG-GTCAGCGATGCTACTGCTTTGGGTACTTACAGGACCCGTCTTG.

16S sequences (GenBank accession number: [ON059710.1](#));

TCCCCTGTTTATCAAAAACATGTCTTATTGAAAATAATTTTAAGT-CAAATCTGCTCAATGATAATTTTATTAAATAGCTGCAGTAAATATGACT-GTACTAAGGTAGCATAATAAATAGTTTATTAATTATAAACTTGTATGAAA-GATTTAATGTAATAAATACTGTTTCAATTTAAAAAAATAAATTTTTTTTTT-TAAGTAAAAAACTTAAATAAAATTTAAAGACGAGAAGACCCTATA-GAATTTTATAAATTAATTTTAATTAATTTATTTTAAATTAATAATTAAT-TATTTAATTGGGGAGATTATAAAATTTAAAAAACTTTTATATAAATTTA-CAATAATTATTGAATAAAATATAATTTTTTAAAAAAATAAAATAAATTACCT-TAGGGATAACAGCATAATTTTTTTTTTTAAGTTCGTATTACTAAAAAAGAT-TATGACCTCGATGTTGAATTAAGA

**Description.** Body  $\sim 9.35$  mm. **Head:** Antenna 40-segmented. Face width  $\sim 1.73 \times$  longer than its height (1.28:0.74). Width of anterior ocellus  $\sim 1.15 \times$  longer than POL (0.15:0.13). Eyes bulged and without interommatidial setae; median width of eye about  $\sim 1.04 \times$  longer than median width of gena in lateral view (0.58:0.56). Clypeus  $\sim 2.39 \times$  longer than its height (0.74:0.31). **Mesosoma:** Notauli weakly impressed at anterior half and absent posteriorly. Scutellar sulcus weakly impressed, medially shallow, laterally relatively deep, without crenula. Pronotum mostly smooth, with ventral longitudinal carina. Metapleuron mostly smooth. Propodeum  $\sim 0.39 \times$  longer than its median width (0.66:1.68); median longitudinal furrow present, elongate isosceles trapezoid, anteriorly opened posteriorly closed by nucha, anterior width  $\sim 0.19 \times$  longer than maximum width (0.03:0.16). **Legs:** Basal spur on mid tibia  $\sim 0.71 \times$  longer than length of basitarsus (0.62:0.87). Basal spur on hind tibia  $0.50 \times$  longer than length of basitarsus (0.84:1.68); claw pectinate. **Wings:** Fore wing (RS+M)a vein present; second submarginal cell trapezoid; 1r absent; 3r absent; 3RSb evenly curved. Hind wing





**Figure 8.** *Neocardiochiles victoriae* sp. nov. **A** lateral habitus **B** dorsal habitus **C** anterior head **D** hypopygium **E** dorsal propodeum and T1–T3.

2r-m absent; 2–1A absent. **Metasoma:** T1  $\sim 1.39 \times$  longer than its posterior width (1.42:1.02), anterior width  $\sim 0.56 \times$  longer than posterior width (0.57:1.02), dorsally isosceles trapezoid; Y-shaped suture present. T2 nearly oval,  $\sim 0.39 \times$  longer than its posterior width (0.49:1.27), with curved posterior margin. Hypopygium with median fold. Ovipositor sheath slightly downcurved,  $\sim 1.04 \times$  longer than length of hind tibia (3.38:3.25), evenly setose except for base.

**Color.** Body mostly orange. The following areas dark: antenna (reddish brown), head, ventral fore notum, fore coxa, fore trochanter and trochantellus (mostly), fore femur (mostly); middle coxa, middle trochanter and trochantellus (mostly), middle femur (mostly), mid tibia (apically); hind femur (apically); hind tibia (except for inner tibia), fifth to last terga (dorsally), ovipositor sheath. Fore wing with two bands; stigma mostly pale.

**Etymology.** Named in honor of Ms Victoria Bayless, a curator in the Louisiana State Arthropod Museum at LSU AgCenter and a former president of the Coleopterists Society, who is the best friend in LSU and has red hair as the specimen has reddish antennae.

**Male.** Unknown.

**Host.** Unknown.

**Distribution.** *Neocardiochiles victoriae* sp. nov. is known only from one female specimen collected from Degrad Laurens, Crique Sapokai, French Guiana.

***Neocardiochiles whitfieldi* (Mercado, 2003), comb. nov.**

**Material examined.** **Holotype** MEXICO • ♂; 16 km north of Autlan, Jalisco; 7.vii.1984; Carrol, Schaffner, Friedlander. Deposited in TAMU.

**Diagnosis.** The known male of *Neocardiochiles whitfieldi* can be easily distinguished from other members of *Neocardiochiles* by the following color characters: head and mesosoma darker; wings entirely melanic; metasoma orange.



**Description (Male).** See Mercado and Wharton (2003).

**Female.** Unknown.

**Host.** Unknown.

**Distribution.** Mexico.

## Acknowledgements

We thank Drs Daniel Janzen and Winnie Hallwachs at the University of Pennsylvania, Dr Michael Sharkey at HIC, Dr Scott Shaw at UWIM, Dr Karen Wright at TAMU, Dr Tommy McElrath at INHS, Dr Zoltan Vas at HNHM, and Mr Yves Braet in Belgium for loan of specimens and museum research. We also thank Société Entomologique Antilles-Guyane (S.E.A.G) and Association Entomologique des Inselbergs de Guyane (A.E.I. Guyane) for their massive collecting and sorting efforts in French Guiana. In addition, we are grateful to Drs Chris Carlton, Michael Stout, Claudia Husseneder, and Rodrigo Diaz in the Department of Entomology at LSU AgCenter for invaluable advice and supports. Also, the first and second authors would like to acknowledge Ms Victoria Bayless, curator in Louisiana State Arthropod Museum for her encouragement and friendship. In addition, we are grateful to Dr Scott Herke at the LSU Genomics Facility for his help and work. Finally, we thank the LSU Agricultural Center and LSU Entomology Department for financial and equipment support.

## References

- Belshaw R, Lopez-Vaamonde C, Degerli N, Quicke DL (2001) Paraphyletic taxa and taxonomic chaining: evaluating the classification of braconine wasps (Hymenoptera: Braconidae) using 28S D2–3 rDNA sequences and morphological characters. *Biological Journal of the Linnean Society* 73: 411–424. <https://doi.org/10.1111/j.1095-8312.2001.tb01370.x>
- Belshaw R, Quicke DL (1997) A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Molecular Phylogenetics Evolution* 7(3): 281–293. <https://doi.org/10.1006/mpev.1996.0400>
- Brullé A (1846) *Histoire Naturelles des Insects: Hymenopteres*. Tome quatrieme. Librairie Encyclopedique de Roret, Paris, 689 pp. <https://doi.org/10.5962/bhl.title.9005>
- Campbell B, Heraty J, Rasplus JY, Chan K, Steffen-Campbell J, Babcock C (2000) Molecular systematics of the Chalcidoidea using 28S-D2 rDNA. *Hymenoptera evolution, biodiversity and biological control*. CSIRO Publishing, Collingwood, Australia 26: 59–73.
- Chen YI, Pike KS, Greenstone MH, Shufran KA (2006) Molecular markers for identification of the hyperparasitoids *Dendrocercus carpenteri* and *Alloxysta xanthopsis* in *Lysiphlebus testaceipes* parasitizing cereal aphids. *Biocontrol* 51(2): 183–194. <https://doi.org/10.1007/s10526-005-1518-0>
- Dabek EZ, Whitfield JB, Hallwachs W, Janzen DH (2020) Two new reared species of *Heteropteron* Brullé (Hymenoptera, Braconidae, Cardiochilinae) from northwest Costa Rica,



- with the first definitive host records for the genus. *Journal of Hymenoptera Research* 77: 151–165. <https://doi.org/10.3897/jhr.77.50577>
- Dallwitz MJ, Paine TA, Zurcher EJ (1999) User's guide to the DELTA Editor. <http://delta-intkey.com> [accessed on Mar. 12. 2022]
- Dallwitz MJ, Paine TA, Zurcher EJ (2000) Principles of interactive keys. <http://delta-intkey.com> [accessed on Mar. 12. 2022]
- Dangerfield PC, Austin AD, Whitfield JB (1999) Systematics of the world genera of Cardiochilinae (Hymenoptera: Braconidae). *Invertebrate Systematics* 13(6): 917–976. <https://doi.org/10.1071/IT98020>
- Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37(1): 291–294. <https://doi.org/10.1093/molbev/msz189>
- Dowton M, Austin AD (1994) Molecular phylogeny of the insect order Hymenoptera: apocritan relationships. *Proceedings of the National Academy of Sciences of the United States of America* 91(21): 9911–9915. <https://doi.org/10.1073/pnas.91.21.9911>
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Edler D, Klein J, Antonelli A, Silvestro D (2021) raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12(2): 373–377. <https://doi.org/10.1111/2041-210X.13512>
- Fagan-Jeffries EP, Austin AD (2020) Synopsis of the parasitoid wasp genus *Cotesia* Cameron, 1891 (Hymenoptera: Braconidae: Microgastrinae) in Australia, with the description of seven new species. *European Journal of Taxonomy* 667: 1–70. <https://doi.org/10.5852/ejt.2020.667>
- Fernandez-Triana J, Whitfield J, Rodriguez J, Smith M, Janzen D, Hajibabaei M, Burns J, Solis A, Brown J, Cardinal S, Goulet H, Hebert P (2014) Review of *Apanteles* sensu stricto (Hymenoptera, Braconidae, Microgastrinae) from Area de Conservación Guanacaste, northwestern Costa Rica, with keys to all described species from Mesoamerica. *ZooKeys* 383: 1–565. <https://doi.org/10.3897/zookeys.383.6418>
- Fernandez-Triana J, Boudreault C, Dapkey T, Smith MA, Hallwachs W, Janzen D (2019) A revision of *Dolichogenidea* (Hymenoptera, Braconidae, Microgastrinae) with the second mediotergite broadly rectangular from Area de Conservación Guanacaste, Costa Rica. *ZooKeys* 835: 87–123. <https://doi.org/10.3897/zookeys.835.33440>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Harris RA (1979) Glossary of surface sculpturing. *Occasional Papers in Entomology* 28: 1–31. <https://doi.org/10.5281/zenodo.26215>
- Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22(2): 160–174. <https://doi.org/10.1007/BF02101694>
- Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*.



- Proceedings of the National Academy of Sciences of the United States of America 101(41): 14812–14817. <https://doi.org/10.1073/pnas.0406166101>
- Kang I, Chapman EG, Janzen DH, Hallwachs W, Dapkey T, Smith MA, Sharkey MJ (2017) Revision of the species of *Lytopylus* from Area de Conservación Guanacaste, northwestern Costa Rica (Hymenoptera, Braconidae, Agathidinae). ZooKeys 721: 93–158. <https://doi.org/10.3897/zookeys.721.20287>
- Maddison WP, Maddison DR (2021) Mesquite: a modular system for evolutionary analysis. Version 3.70. <http://www.mesquiteproject.org> [accessed on Oct. 10. 2021]
- Madeira F, mi Park Y, Lee J, Buso N, Gur T, Madhusoodanan N, Basutkar P, Tivey ARN, Potter SC, Finn RD, Lopez R (2019) The EMBL-EBI Search and Sequence Analysis Tools APIs in 2019. Nucleic Acids Research 47: W636–W641. <https://doi.org/10.1093/nar/gkz268>
- Meierotto S, Sharkey MJ, Janzen DH, Hallwachs W, Hebert PDN, Chapman EG, Smith MA (2019) A revolutionary protocol to describe understudied hyperdiverse taxa and overcome the taxonomic impediment. Deutsche Entomologische Zeitschrift 66(2): 119–145. <https://doi.org/10.3897/dez.66.34683>
- Mercado I, Wharton RA (2003) Mexican cardiochiline genera (Hymenoptera: Braconidae), including a preliminary assessment of species-groups in *Toxoneuron* Say and *Retusigaster* Dangerfield, Austin and Whitfield. Journal of Natural History 37(7): 845–902. <https://doi.org/10.1080/00222930110097167>
- Murphy N, Banks JC, Whitfield JB, Austin AD (2008) Phylogeny of the microgastroid complex of subfamilies of braconid parasitoid wasps (Hymenoptera) based on sequence data from seven genes, with an improved estimate of the time of origin of the lineage. Molecular Phylogenetics and Evolution 47: 378–395. <https://doi.org/10.1016/j.ympev.2008.01.022>
- Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, Oxford, 352 pp.
- Papp J (2014) A revisional study on Szépligeti's cardiochiline type specimens deposited in the Hungarian Natural History Museum, Budapest (Hymenoptera, Braconidae: Cardiochilinae). In Annales historico-naturales Musei nationalis hungarici 106: 169–214.
- Sharkey MJ, Wharton RA (1997) Morphology and terminology. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) Manual of the New World genera of the family Braconidae (Hymenoptera). Special Publication of the International Society of Hymenopterists, No 1, Washington DC, 19–37.
- Sharkey MJ, Janzen DH, Hallwachs W, Chapman EG, Smith MA, Dapkey T, Brown A, Ratnasingham S, Naik S, Manjunath R, Perez K, Milton M, Hebert P, Shaw SR, Kittel RN, Solis MA, Metz MA, Goldstein PZ, Brown JW, Quicke DLJ, van Achterberg C, Brown BV, Burns JM (2021a) Minimalist revision and description of 403 new species in 11 subfamilies of Costa Rican braconid parasitoid wasps, including host records for 219 species. ZooKeys 1013: 1–665. <https://doi.org/10.3897/zookeys.1013.55600>
- Sharkey MJ, Baker A, McCluskey K, Smith A, Naik S, Ratnasingham S, Manjunath R, Perez K, Sones J, D'Souza M, Jacques BS, Hebert P, Hallwachs W, Janzen D (2021b) Addendum to a minimalist revision of Costa Rican Braconidae: 28 new species and 23 host records. ZooKeys 1075: 77–136. <https://doi.org/10.3897/zookeys.1075.72197>
- Slater-Baker MR, Austin AD, Whitfield JB, Fagan-Jeffries EP (2022) First record of miracine parasitoid wasps (Hymenoptera: Braconidae) from Australia: molecular phylogenetics and



- morphology reveal multiple new species. *Austral Entomology* 61(1): 49–67. <https://doi.org/10.1111/aen.12582>
- Smith MA, Rodriguez JJ, Whitfield JB, Deans AR, Janzen DH, Hallwachs W, Hebert PD (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences* 105(34): 12359–12364. <https://doi.org/10.1073/pnas.0805319105>
- Smith MA, Fernández-Triana JL, Eveleigh E, Gómez J, Guclu C, Hallwachs W, Hebert PDN, Hrcek J, Huber JT, Janzen DH, Mason PG, Miller S, Quicke DLJ, Rodriguez JJ, Rougerie R, Shaw MR, Várkonyi G, Ward D, Whitfield JB, Zaldivar-Riveron A (2012) DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): impacts after 8 years and nearly 20,000 sequences. *Molecular Ecology Resources* 13(2): 168–176. <https://doi.org/10.1111/1755-0998.12038>
- Szépligeti V (1908) Braconiden aus der Sammlung des Ungarischen National-Museums. II Teil. *Annales Historico-Naturales Musei Nationalis Hungarici* 6: 397–427.
- Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* 38(7): 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Whitfield JB, Dangerfield PC (1997) Subfamily Cardiochilinae. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) *Manual of the New World Genera of the Family Braconidae* (Hymenoptera). International Society of Hymenopterists, Washington DC, 177–183.
- Yu DS, van Achterberg CV, Horstmann K (2016) Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive. Ottawa, Ontario.

## Supplementary material I

**Maximum likelihood (ML) phylogeny based on 16S data Maximum likelihood (ML) phylogeny based on 16S data indicating the relationships of *Neocardiochiles chriscarltoni* sp. nov. and *N. victoriae* sp. nov. *Protomicroplitis calliptera* (Hymenoptera: Braconidae: Microgastrinae) is included as the outgroup.**

Authors: Ilgoo Kang, James Whitfield, Brittany Owens, Junyan Chen

Data type: Image

Explanation note: Maximum likelihood (ML) phylogeny based on 16S data indicating the relationships of *Neocardiochiles chriscarltoni* sp. nov. and *N. vitoriae* sp. nov. *Protomicroplitis calliptera* (Hymenoptera: Braconidae: Microgastrinae) is included as the outgroup.

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## Supplementary material 2

**Maximum likelihood (ML) phylogeny based on 28S data Maximum likelihood (ML) phylogeny based on 28S data indicating the relationships of *Neocardiochiles chriscarltoni* sp. nov. and *N. victoriae* sp. nov. *Protomicroplitis calliptera* (Hymenoptera: Braconidae: Microgastrinae) is included as the outgroup.**

Authors: Ilgoo Kang, James Whitfield, Brittany Owens, Junyan Chen

Data type: Image

Explanation note: Maximum likelihood (ML) phylogeny based on 28S data indicating the relationships of *Neocardiochiles chriscarltoni* sp. nov. and *N. vitoriae* sp. nov. *Protomicroplitis calliptera* (Hymenoptera: Braconidae: Microgastrinae) is included as the outgroup.

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## Supplementary material 3

***Neocardiochiles*\_DELTA\_Key**

Authors: Ilgoo Kang, James Whitfield, Brittany Owens, Junyan Chen

Data type: Delta interactive key

Explanation note: Files and sources of DELTA Interactive Key.

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## Supplementary material 4

### **Character matrix for *Heteropteron*, *Wesmaelella*, and *Neocardiochiles* (Hymenoptera: Braconidae: Cardiochilinae)**

Authors: Ilgoo Kang, James Whitfield, Brittany Owens, Junyan Chen

Data type: Nexus File

Explanation note: Character matrix for *Heteropteron*, *Wesmaelella*, and *Neocardiochiles* (Hymenoptera: Braconidae: Cardiochilinae) used in phylogenetic analysis.

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